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CLAIMS

A process for reducing the fluorescence quenching caused by the measuring medium, in a fluorescence assay for an analyte using at least one fluorescent label, characterized in that a fluorescent conjugate comprising an oligonucleotide bonded to a rare-earth metal cryptate is introduced into the measuring medium.

- 2. The process as claimed in claim 1, characterized in that the oligonucleotide consists of a chain of ribonucleotide or deoxyribonucleotide units bonded to one another via phosphodiester-type bonds.
- 3. The process as claimed in claim 1, characterized in that the oligonucleotide consists of a chain of ribonucleotide or deoxyribonucleotide units or of analogous units of nucleotides modified on the sugar or on the base and bonded to one another via natural phosphodiester-type internucleotide bonds, some of the internucleotide bonds optionally being replaced with phosphonate, phosphoramide or phosphorothioate bonds.
- 4. The process as claimed in claim 1, characterized in that the oligonucleotide consists of a chain comprising both ribonucleotide or deoxyribonucleotide units bonded to one another via phosphodiester-type bonds and analogous units of nucleosides bonded to one another via amide bonds.
- 5. The process as claimed in any one of claims 1 to 4, characterized in that the oligonucleotide consists of ribonucleotide or deoxyribonucleotide units, one of which may comprise a functional group introduced onto or generated on said unit, or a functional group introduced using a spacer arm bonded to the terminal phosphate group in the 8' or 5' position.
 - 6. The process as claimed in claim 5, characterized in that said unit is the 5' terminal unit or 3' terminal unit.
- 7. The process as claimed in any one of claims 1 to 6, characterized in that the oligonucleotide comprises a chain of 5 to 50 nucleotides or a chain of 5 to 30 50 nucleotides and nucleotide or nucleoside analogs.
 - 8. The process as claimed in any one of claims 1 to 6, characterized in that the oligonucleotide consists of a chain of ribonucleotide or deoxyribonucleotide units bonded to one another via phosphodiester-type bonds and of analogous units of nucleosides bonded to one another via amide bonds, said oligonucleotide

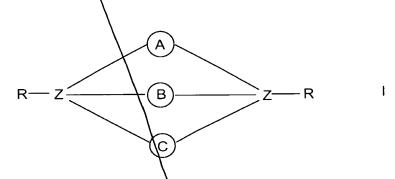
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comprising at least 5 phosphodiester-type internucleotide bonds at the end intended to be bonded to the cryptate.

- 9. The process as claimed in any one of claims 1 to 8, characterized in that the rare-earth netal cryptate is bonded covalently to the oligonucleotide either directly or via a space; arm.
- 10. The process as claimed in any one of claims 1 to 9, characterized in that said rare-earth metal cryptate consists of at least one rare-earth metal salt complexed with a macropolycyclic compound of formula



in which Z is an atom with 3 or 4 valencies, R is nothing or represents hydrogen, a hydroxy group, an amino group or a hydrocarbon-based radical, the divalent radicals (A), (B) and (C), are, independently of each other, hydrocarbon-based chains which optionally contain one or more hetero atoms and are optionally interrupted with a hetero macrocycle, at least one of the radicals (A), (B) and (C), also comprising at least one molecular unit or consisting essentially of a molecular unit, said molecular unit having a triplet energy which is

11. The process as claimed in claim 10, characterized in that the rareearth metal cryptate consists of a rare-earth metal salt complexed with one of the macrocyclic or macropolycyclic compounds below:

greater than that of the emission level of the complexed rare-earth metal ion.

(22)phenanthroline; (22)phenanthrolinamide; (22)anthracene; (22)anthracenamide; (22)biisoquinoline; (22)biphenylbispyridine; (22)bipyridine; (22)bipyridine, trisphenanthroline, phenanthrolinebisbipyridine, biisoquinolinebisbipyridine, bisbipyridine diphenylbipyridine; a macropolycyclic compound comprising a molecular unit

chosen from bipyrazines, bipyrimidines and nitrogen-containing heterocycles comprising N-oxide groups.

The process according to any one of claims 1 to 9, characterized in that the rare earth metal cryptate consists of at least one rare-earth metal salt complexed with a macropolycyclic compound corresponding to one of the formulae II or III below:

is one of the following rings:

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R'

n = 0 or 1

[N₂O₄] macrocycle or cycle (22) [N₂O₃] macrocycle or cycle (21)

bisbipyridine macrocycle

- Y is a spacer group or spacer arm which consists of a divalent organic radical, chosen from linear or branched C_1 to C_{20} alkylene groups optionally containing one or more double bonds and/or optionally containing one or more hetero atoms such as oxygen, nitrogen, sulfur or phosphorus or one or more carbamoyl or carboxamido group(s); chosen from C_5 to C_8 cycloalkylene groups or chosen from C_6 to C_{14} arylene groups, said alkylene, cycloalkylene or arylene groups being optionally substituted with alkyl, aryl or sulfonate groups;
- Z is a functional group capable of bonding covalently to a biological substance;
 - R is a methyl group or represents the group -Y-Z;
- R' is hydrogen or a group -COOR" in which R" is a C₁ to C₁₀ alkyl group and preferably represents a methyl, ethyl or tert-butyl group, or alternatively R' is a group -CO-NH-Y-Z.
- 13. The process as claimed in any one of claims 1 to 12, characterized in that the rare-earth metal cryptate is bonded to the oligonucleotide via a spacer arm consisting of a divalent organic radical chosen from C_1 - C_{20} linear or branched alkylene groups optionally containing one or more double bonds or triple bonds

and/or optionally containing one or more hetero atoms, such as oxygen, nitrogen, sulfur, phosphorus or one or more carbamoyl or carboxamido group(s); C_5 - C_8 cycloalkylene groups and C_6 - C_{14} arylene groups, said alkylene, cycloalkylene or arylene groups being optionally substituted with alkyl, aryl or sulfonate groups.

14. The process as claimed in claim 13, characterized in that the spacer armus chosen from the groups:

$$-CONH$$
 $S-(CH_2)_n$
 $S-(CH_2)_n$
 $S-(CH_2)_n$

in which n = 2 to 6, and -CONH-(CH₂)₆-, the attachment via the group -CONH taking place on the cryptate.

10 July 15. The method as claimed in any one of claims 1 to 14, characterized in that the rare-earth metal cryptate is a europium cryptate.

16. The process as claimed in claim 15, characterized in that the rareearth metal cryptate is the europium cryptate Eu trisbipyridine or Eu [bisdiethoxybipyridine.bipyridine].

- 15 / 17. The process as claimed in any one of claims 1 to 16, characterized in that the fluorescent conjugate is used as the only label or as one of the fluorescent labels in the assay.
 - 18. The process as claimed in any one of claims 1 to 17, characterized in that the fluorescent conjugate is bonded covalently to one of the members of a pair of molecules capable of binding specifically to one another, in particular a cellular receptor, an antigen, an antibody or a nucleic acid.
 - 19. The process as claimed in any one of claims 1 to 18, characterized in that, in addition to said fluorescent conjugate, a fluorescent label comprising an acceptor fluorescent compound is used in the assay.